Development of a Genetic System in Siberian Permafrost Isolate, *Psychrobacter* sp. 273-4

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Understanding the low-temperature limits of terrestrial life has a major impact on understanding where in our predominantly cold solar system Earth-like life may exist. We are studying cold-adapted microorganisms from Siberian permafrost to identify the genetic adaptations that allow survival of long-term, low-temperature conditions. In pursuit of this goal, we seek to develop a site-directed mutagenesis system for use in *Psychrobacter* sp. 273-4. We used the suicide plasmid pJK100 for targeted gene deletion (by J. Klappenbach, Michigan State University). The regions upstream and downstream of the general stress protein Ctc were PCR amplified from *Psychrobacter* sp. 273-4 and inserted into multiple cloning sites upstream and downstream, respectively, of the kanamycin resistance gene. The resulting plasmid was introduced into *Psychrobacter* sp. 273-4 via conjugation and potential mutants were selected for on media containing kanamycin. Following conjugation, ~69% of the potential mutants screened had the desired phenotype that indicated a double recombination event had occurred between plasmid and genomic DNA. Double recombination was confirmed via successful PCR amplification of both the upstream and downstream genome-plasmid junctions. However, when PCR amplification was performed with primers immediately up- and down-stream of the deletion site to confirm that the targeted gene had been replaced by the kanamycin resistance gene, both the targeted gene and the kanamycin resistance gene were amplified. These results suggest that *Psychrobacter* sp. 273-4 contains a polyploid genome. Continued investigations will verify the ploidy of the *Psychrobacter* sp. 273-4 genome and determine how to obtain homozygous mutants in *Psychrobacter* sp. 273-4.